

and peninsulae corresponding to preserved cortex. There was variable preservation of the cerebrum, total liquefaction of occipital lobes, and irregular preservation of the outer layers of some parts of the temporal and frontal lobes. Altogether, the picture was compatible with severe porencephaly or hydranencephaly. The spine showed no sign of scoliosis, and movement of the limb joints was not restricted (i.e., no arthrogryposis).

Samples were removed from the remnants of the cerebrum, diencephalon, and organs (thymus, lung, myocardium, jejunum, ileum, mesenteric lymph node, liver, spleen, kidney, and striated muscle), and 3 independent real-time PCR protocols were conducted to detect genomes of bovine viral diarrhea/mucosal disease virus, bluetongue virus serotype 8, and the novel SBV. Initial retrotranscription of the RNA genomes was followed by quantitative (real-time) PCR. The process was conducted by using our procedures (2) and, for SBV, by following the protocol and using recently developed control reagents as described (1). The SBV genome was detected in only CNS samples (quantification cycle value 28.8); bovine viral diarrhea/mucosal disease virus and BTV-8 genomes were not detected. The new virus genome load was  $1.61 \times 10^4$  copies per gram of cerebrum sample.

Taken together, the above data suggest that, like other Simbu serogroup viruses, the new virus crosses the placenta, contaminates the bovine fetus, infects the fetus' CNS, and causes necrosis and/or developmental arrest of the cerebral cortex. Unlike the viruses mentioned above (3,4), and provided this case is not an exception, the SBV genome seems to persist in the infected fetus and is detectable after birth by real-time reverse transcription PCR, despite gestation length. Although reliable reagents for detecting seroconversion are temporarily unavailable, the persistence of the

new virus in fetal tissue should greatly facilitate the epidemiologic monitoring of the emergence and spread of the new virus.

When calves from experimentally infected dams are infected with the closest phylogenetic relative to SBV, Akabane virus, porencephaly develops during gestational days 62–96 (5). If the same is true for the new virus, the above calf was probably infected during June 9–July 13, 2011. Therefore, it is hypothesized that infected arthropods were already circulating in the village of Hamois-in-Condroz (50°24'56"N, 5°8'7"E), which is  $\approx 240$  km southwest of Schmallenberg (51°8'42"N, 8°17'18"E),  $\approx 2$  months before the emergence of the clinical syndrome that led to the identification of the new virus.

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## Zoonotic Disease Pathogens in Fish Used for Pedicure

**To the Editor:** Doctor fish (*Garra rufa*) are freshwater cyprinid fish that naturally inhabit river basins in central Eurasia. They are widely used in the health and beauty industries in foot spas for ichthyotherapy (Kangal fish therapy or doctor fish therapy) (Figure; online Technical Appendix Figure 1, [wwwnc.cdc.gov/EID/pdfs/11-1782-Techapp.pdf](http://wwwnc.cdc.gov/EID/pdfs/11-1782-Techapp.pdf)) (1). During these sessions, patients immerse their feet or their entire bodies in the spas, allowing the fish to feed on dead skin for cosmetic reasons or for control of psoriasis, eczema, and other skin conditions.

A survey during the spring of 2011 identified 279 fish spas in the United Kingdom, and the number has probably increased since then (1). The Fish Health Inspectorate of the Centre for Environment, Fisheries & Aquaculture Science estimates that each week 15,000–20,000 *G. rufa* fish are imported from Indonesia and

other countries in Asia into the United Kingdom through London Heathrow Airport (the main border inspection post for the import of live fish). However, ichthyotherapy has now reportedly been banned in several US states and Canada provinces because of sanitary concerns (1). In the United Kingdom, a limited number of infections after fish pedicures have been reported (1). Unfortunately, little is known about the types of bacteria and other potential pathogens that might be carried by these fish and the potential risks that they might pose to customers or to ornamental and native fish.

On April 12, 2011, the Fish Health Inspectorate investigated a report of a disease outbreak among 6,000 *G. rufa* fish from Indonesia that had been supplied to UK pedicure spas. Affected fish showed clinical signs of exophthalmia and of hemorrhage around the gills, mouth, and abdomen. More than 95% of the fish died before the remaining fish were euthanized. Histopathologic examinations identified systemic bacterial infections with small gram-positive cocci, mostly in the kidneys, spleen, and liver. Bacterial isolates cultured from affected fish were identified as *Streptococcus agalactiae* (group B *Streptococcus*) according to a combination of biochemical test results (API Strep; bioMérieux, Marcy l'Étoile, France), Lancefield grouping with serotype B (Oxoid Limited, Basingstoke, UK), and molecular (partial 16S rRNA gene sequencing) testing methods.

Multilocus sequence typing of a representative isolate (11013; online Technical Appendix Table) (2) indicated that it was a sequence type (ST) 261 *S. agalactiae* strain (<http://pubmlst.org/sagalactiae>). This same ST261 profile was first identified in an isolate (ATCC 51487) from a diseased tilapia in Israel (3). The clinical appearance of the disease and the diagnostic results suggested that *S.*

*agalactiae* was the causative agent of the fish illness and deaths.

To determine whether *S. agalactiae* and other bacterial pathogens might be carried more widely by these fish, from May 5, 2011, through June 30, 2011, the Fish Health Inspectorate of the Centre for Environment, Fisheries & Aquaculture Science visited Heathrow Airport 5 times to intercept and sample consignments of *G. rufa* from Indonesia. A taxonomically diverse range of bacteria were identified (online Technical Appendix Table, Figure 2), including a variety of human pathogens capable of causing invasive soft tissue infections. These pathogens included *Aeromonas* spp. (4), potentially pathogenic clinical-type *Vibrio vulnificus* isolates (online Technical Appendix Figure 2) (5), non-serotype O1 or O139 cholera toxin-negative *V. cholerae* isolates (online Technical Appendix Figure 2) (6), *Mycobacteria* (7), and *S. agalactiae* (3,8). Isolates were resistant to a variety

of antimicrobial drugs, including tetracyclines, fluoroquinolones, and aminoglycosides (online Technical Appendix Table). Other studies have also reported high levels of multidrug resistance in bacteria associated with imported ornamental fish (9).

Water is a well-recognized source of bacterial skin infections in humans. *V. vulnificus* can cause wound infections and primary septicemia, resulting in high mortality rates, especially among persons with predisposing risk conditions (e.g., liver disease, diabetes, or impaired immune function) (5). *S. agalactiae* is a common cause of skin and soft tissue infections, especially in older adults and those with chronic diseases such as diabetes mellitus (8). Although *S. agalactiae* ST261 is not considered to be one of the genotypes typically associated with invasive disease in humans (3), a fish-adapted strain could eventually take advantage of the opportunity afforded by repeated exposure and thereby also affect



Figure. Doctor fish surrounding foot during ichthyotherapy.

humans. Additionally, *Mycobacteria* spp. can occasionally cause disease in humans through contact with fish (*M. marinum*), and pedicure treatments have previously been associated with *M. fortuitum* infections (10).

Recently, the risks associated with exposure to *G. rufa* fish were reported to be low (1). To date, there are only a limited number of reports of patients who might have been infected by this exposure route (1). However, our study raises some concerns over the extent that these fish, or their transport water, might harbor potential zoonotic disease pathogens of clinical relevance. In particular, patients with underlying conditions (such as diabetes mellitus or immunosuppression) should be discouraged from undertaking such treatments, especially if they have obvious breaks in the skin or abrasions. This risk can probably be reduced by use of certified disease-free fish reared in controlled facilities under high standards of husbandry and welfare.

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## *Rickettsia conorii* Indian Tick Typhus Strain and *R. slovaca* in Humans, Sicily

**To the Editor:** Rickettsiae are vector-borne pathogens that affect humans and animals worldwide (1). Pathogens in the *Rickettsia conorii* complex are known to cause Mediterranean spotted fever (MSF) (*R. conorii* Malish strain), Astrakhan fever (*R. conorii* Astrakhan strain), Israeli spotted fever (*R. conorii* Israeli spotted fever strain), and Indian tick typhus (*R. conorii* Indian tick typhus strain) in the Mediterranean basin and Africa, southern Russia, the Middle East, and India and Pakistan, respectively (2). These rickettsioses share some clinical features, such as febrile illness and generalized cutaneous rash, and are transmitted to humans by *Rhipicephalus* spp. ticks (2).

MSF is endemic to Sicily (Italy); fatal cases occur each year, and the prevalence of *R. conorii* in dogs is high (3–6). Recently, *R. conorii* Malish strain and *R. conorii* Israeli spotted fever strain were confirmed in humans in Sicily in whom MSF was diagnosed (4), which suggests that other *R. conorii* strains might be present and diagnosed as causing MSF. The rickettsiae within the *R. conorii* complex, which are relevant for the study of bacterial evolution and epidemiology, can be properly identified only by appropriate genetic analyses.



# Zoonotic Disease Pathogens in Fish Used for Pedicure

## Technical Appendix

Technical Appendix Table. Identities, source, and antimicrobial drug resistance profiles of bacterial isolates from *Garra rufa* fish shipments from Indonesia, sampled at Heathrow Airport, London, UK, May 5, 2011–June 30, 2011. Antimicrobial drug susceptibility was determined for the isolates against 28 antimicrobial drugs from 13 structural classes, using broth-microdilution assays (Sensititer AVIAN and GN2 panels; Trek Diagnostic Systems, East Grinstead, UK). Testing followed guidelines from the Clinical and Laboratory Standards Institute, as described (1).

Isolate	Identity‡ (closest RDP sequence; S <sub>ab</sub> §)	Date, 2011	Resistance phenotype¶
11016	<i>Aeromonas veronii</i> (X60414; 1.00)¶	May 5	(Enr), Otc, Tet, Amx, Pen, Fep, (Ami), (Str), (Spe), Cip, Gat
11040	<i>Aeromonas</i> spp. (X74674; 1.00)¶	May 5	Enr, Tio, Otc, Tet, Amx, Pen, (Ffn), Str, Spe, Faz, (Fur), Cip, Gat, Tob
11021	<i>A. veronii</i> † (X60414; 0.99)¶	May 9	Enr, Otc, Tet, Pen, (Str), Tob
11052	<i>Enterobacteria kobei</i> (AJ508301; 0.951)	Jun 24	Amx, Pen, Faz,
11053	<i>E. kobei</i> (AJ508301; 0.951)	Jun 24	Amx, Pen, Faz,
11038	<i>Citrobacter freundii</i> (AJ233408; 0.977)	Jun 8	Otc, Tet, Pen, (Nit),
11023	<i>Mycobacterium senegalense</i> (AY684063; 100%)§	May 9	Not done
11024	<i>M. senegalense</i> (AY684063; 100%)§	May 9	Not done
11055	<i>Aeromonas</i> spp. (X60408; 0.946)¶	Jun 30	(Otc), (Tet), Pen, (Str),
11056	<i>Aeromonas</i> spp. (X60408; 0.946)¶	Jun 30	Otc, (Tet), Gen
11022	<i>Shewanella</i> spp. (AB205566; 0.934)	May 9	Enr, Otc, Tet, Faz, (Mero), Gen
11037	<i>Shewanella</i> spp. (AB205566; 1.000)	Jun 8	Not Done (isolate lost on subculture)
11013	<i>Streptococcus agalactiae</i> (AB112407; 1.00)	Apr 12	Str, Gen, †
11041	<i>Acinetobacter</i> spp. (AJ293694; 0.905)	Jun 8	Enr, Otc, Tet, (Spe), (Ffn),
11050	<i>Chryseobacterium</i> spp. (EF685359; 0.858)	Jun 24	Enr, Otc, Tet, (Amx), Pen, Str, (Ami), Sxt, Azt, Faz, Tans, Tur, Cip, Gat, Tob, Pod
11054	<i>Chryseobacterium</i> spp. (AM232813; 0.907)	Jun 24	Enr, Tio, Otc, Tet, Amx, (Spe), Str, Ami, Azt, Faz, Tans, Taz, Fur, Cip, (Gat), Gen, Fox, (Tim), Tob, Pod
11011	<i>Vibrio vulnificus</i> (X74727; 1.00)	Apr 12	(Str), (Faz)
11039	<i>V. cholerae</i> (NAG) (X76337; 0.985)	Jun 8	(Otc), (Tet), Amx,
11051	<i>V. vulnificus</i> (X74727; 1.00)	Jun 24	(Spe), Str
11012	<i>V. cholerae</i> (NAG) (X76337; 0.985)	Apr 12	Enr, Otc, Tet, Cip (Gat)
11036	<i>V. mimicus</i> (X74713; 0.975)	May 18	Pen

\*Isolates 11052 and 11053 were from a UK retail outlet; all others were from consignments originating from Indonesia. Isolates represent the range of dominant colony morphotypes observed, recovered from imported *G. rufa* fish. Unless otherwise indicated, all tests reported were performed at 37 ± 2°C and results read after between 24–72 h incubation.

Ami, amikacin; Amx, amoxicillin; Azo, ceftriaxone; Cpr, Ciprofloxacin; Eno, enrofloxacin; Faz, cefazolin; Ffl, florfenicol; Fox, cefoxitin; Fur, cephalothin; Gat, gatifloxacin; Gen, gentamicin; Imi, imipenem; Neo, neomycin; Nit, nitrofurantoin; Pod, cefpodoxime; Str, streptomycin; Pip, piperacillin; Spe, spectinomycin; Str, streptomycin; Sxt, sulfamethoxazole/trimethoprim; Tans, cefotetan Na; Taz, ceftazidime; Tio, ceftiofur; Tob, tobramycin. The range of concentrations of antimicrobial drugs and, in the absence of published break points for the gram-negative organisms tested, the epidemiologic cut-off tolerance values used, were as described (1). Codes in parentheses indicate isolate was of intermediate tolerance to indicated antimicrobial drug.

†Tested at 22°C because growth on Mueller Hinton media at 37°C was poor.

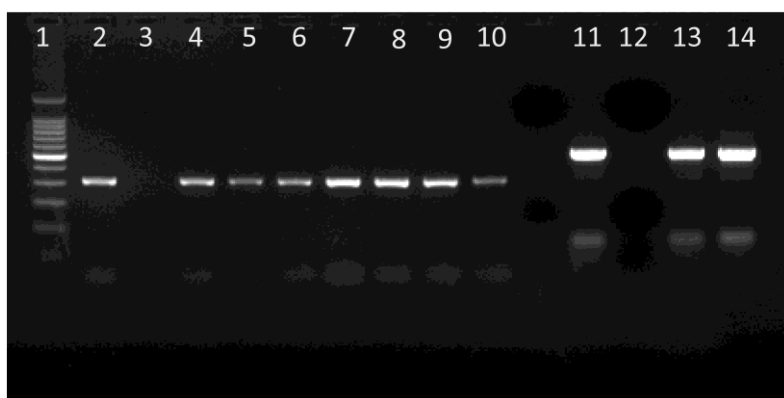
‡All bacterial isolates were identified on the basis of phenotypic testing criteria (Gram stain, cytochrome oxidase and catalase activity and motility), with gram-negative isolates identified further using API 20NE and API 20E (Biomérieux) miniaturized biochemical test systems and partial 16S rRNA gene sequencing. Partial 16S rRNA sequences were aligned to the closest relative using the Ribosomal Database Project SeqMatch tool (<http://rdp.cme.msu.edu/seqmatch/>). Except for isolates 11023 and 11024, closest partial 16S rRNA gene sequence derived from a Type, or otherwise well-characterized strain, displayed and RDP SeqMatch score (S<sub>ab</sub>), with 1.0 representing total sequence identity).

§Species assignment of *Mycobacterium* sp. isolates was based on both partial 16S rRNA and partial RNA polymerase β gene sequencing (2)). Closest RNA polymerase β gene sequence relative obtained by using BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) is displayed.

¶Partial 16S rRNA gene sequencing, species complex assignment of *Aeromonas* spp. isolates were also confirmed based on direct BLAST comparison of partial *gyrA* DNA gene-sequencing (3). Partial 16S and *gyrA* sequences that together shared <95% similarity with a well-characterized strain the organism are listed as an *Aeromonas* spp. only.



Technical Appendix Figure 1. Doctor fish surrounding foot during ichthyotherapy.



Technical Appendix Figure 2. Agarose gel electrophoresis image of *Vibrio* spp.–specific PCR analyses specific for the 310-bp *pRVC* gene fragment in *V. cholerae* (4) and the 519-bp *vvHA* gene fragment in *V. vulnificus* (5). Lane 1, 100-bp DNA ladder (Invitrogen, Carlsbad, CA, USA). Lane 2, positive-control material (*V. cholerae* National Collection of Type Cultures [NCTC] strain 8042). Lane 3, negative control (water), Lanes 4–10, 7 presumptive *V. cholerae* strains isolated on thiosulfate–citrate–bile salts sucrose agar plates and confirmed by PCR. Lane 11, *V. vulnificus*–positive control material (NCTC11067). Lane 12, negative control (water). Lanes 13 and 14, *V. vulnificus* strains 20 and 129.

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